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Pharmacognostical standardization and preliminary phytochemical investigations on *Acacia auriculiformis* A. Cunn. Ex. Benth stem bark

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Abstract
Acacia auriculiformis A. Cunn. ex. Benth is a valuable and evergreen tree of family Mimosaceae. The plant is used as a folk medicine to treat aches, sore eyes, inflammation, malaria, skin diseases like itching, allergy and rashes. The stem bark of the plant is used as traditional remedy for treatment of rheumatism. The present study includes determination of various standardization parameters such as morphological characters, microscopic studies, physicochemical evaluation and preliminary phytochemical screening. The morphological studies exhibited the organoleptic and surface characteristics of stem bark. The microscopic study showed the presence of various characteristics of bark like cork, phloem fibers, medullary rays, parenchyma, calcium oxalate crystals and stone cells. Physicochemical constants such as foreign organic matter, extractive values, ash values, loss on drying, swelling index and foaming index were established. Preliminary phytochemical analysis revealed the presence of carbohydrates, phenols, flavonoids, saponins and steroids. Extensive literature survey on this plant revealed that, no information about standardization of this plant was available. Therefore, findings of this study will facilitate quality control and identification of the plant.

Keywords: *Acacia auriculiformis*, pharmacognostical, phytochemical, physicochemical

1. Introduction
Pharmacognostical standardization is an efficient tool to establish quality control parameters of plants. It helps to assure the authentication of plants and prevention of adulteration [1, 2]. These studies also ensure reproducible quality of plant material and herbal products in trade [2]. Standardization and quality control of plants are also essential for the worldwide acceptance of herbal products in modern system of medicines. Hence, each country has adopted a set of guidelines quality control of the herbal medicine [3]. *Acacia auriculiformis* A. Cunn. ex. Benth. (Mimosaceae) is a straight, medium-sized evergreen tree of family Mimosaceae. It is a native plant of Australia which was first introduced to India in 1946 in West Bengal [4]. The plant is used as a folk medicine to treat aches, sore eyes, inflammation, malaria, skin diseases like itching, allergy and rashes. The plant also exhibits various pharmacological activities like antioxidant [5], antimalarial [6], antifilarial [7], cestocidal [8], antimicrobial [9], spermicidal [10], wound healing [11], anti-arthritic [12], antimutagenic and chemopreventive [13], hepatoprotective and anti diabetic activity [14]. Extensive literature survey on this plant revealed that, no information about standardization of this plant was available. Therefore, the present study was undertaken to establish quality control parameters for standardization of the *Acacia auriculiformis* A. Cunn. ex. Benth. stem bark.

2. Material and methods
2.1 Plant material
Acacia auriculiformis A. Cunn. was collected from Guru Jambheshwar University of Science & Technology, Hisar in month of March, 2015. The stem bark of the plant is selected for the proposed work. The plant was authenticated by Dr. Anjula Pandey, Principal scientist, National Herbarium of cultivated plants, NBGPR, New Delhi, vide reference no, NHCP/NBGPR/2015-3 dated 22.04.2015 The plant was identified as *Acacia auriculiformis* A. Cunn. ex. Benth. Family Mimosaceae.

2.2 Morphological studies
The morphological studies were performed by visual examination and with the help of dissection microscope. The morphological characteristics like color, odour, taste, shape, size, texture and surface characteristics were determined.

2.3 Microscopical studies
Transverse section of stem bark was cut by free hand sectioning. The transverse sections were cleared in chloral hydrate with gentle warming. Then, sections were stained with phloroglucinol and concentrated hydrochloric acid. Further, the sections were mounted in glycerin and covered with cover slip. For powder microscopy, small amount of stem bark powder was taken on slide. This powder was stained with phloroglucinol and concentrated hydrochloric acid. Then, it was mounted in glycerin and covered with cover slip. The prepared slides were observed under light microscope (Carl Zeiss Primo star, Germany). Histochemical studies were also done by using various chemical reagents to detect cell wall and its contents in the plant stem bark [15].

2.4 Physicochemical parameters
Physicochemical parameters of the plant stem bark were studied using standard procedures [15, 16]. These parameters include foreign organic matter, loss on drying, extractive values, ash values, swelling index and foaming index.

2.5 Elemental analysis
Elemental analysis of the stem bark powder was done using nitric-perchloric acid digestion method using the procedure recommended by the AOAC (1990). For this analysis, one gram of stem bark powder boiled gently with 10 ml of concentrated nitric acid for 30-45 min. This mixture was cooled down and 5 ml of 70% perchloric acid was added to it. Further, the mixture was boiled gently until the appearance of dense white fumes. This solution was cooled down and 20 ml of distilled water was added to it and boiled to release the white fumes. After cooling, the solution was filtered through whatman No. 42 filter paper [17, 18]. The samples obtained after filtration were analyzed in Atomic Absorption Spectroscopy (AAS) (GBC 932 plus). An atomic absorption spectrophotometer with hollow cathode lamp for lead (Pb), cadmium (Cd), copper (Cu), arsenic (As), zinc (Zn), mercury (Hg), iron (Fe) and magnesium (Mg) was used. The instrument was calibrated by using the standard solutions of As, Hg, Pb, Cd, Cu, Zn, Fe and Mg at various wavelengths 193.7, 253.7, 283.5, 228.8, 324.8, 213.9, 248.3, 242.1 nm respectively. Then, the standard calibration curves of these elements were prepared. The instrument was optimized as per requirement and results were obtained in ppm levels.

2.6 Preliminary phytochemical screening
The powdered stem bark of *Acacia auriculiformis* A. Cunn.ex. Benth. (100g) was defatted by extracting with petroleum ether and then successively extracted with ethyl acetate, ethanol and water. Each time the powdered drug was extracted with one solvent, it was dried below 50º C before extracting with the next solvent. The ethanol extract was prepared by using soxhlet apparatus. The extract obtained from soxhalation was concentrated by distilling off the solvent and recovering the same. The total aqueous extract was prepared by cold maceration method. The drug was macerated with distilled water for 24 hours and then filtered. The marc obtained was again macerated with distilled water and filtered. The filtrates were combined. The ethanol and aqueous extracts were then evaporated to dryness to obtain dried extracts and were kept in dessicator. The prepared extracts were subjected to various chemical tests for the presence of different phytoconstituents [3].

2.7 Determination of total phenol content
The amount of total phenol content in stem bark was determined by using Folin-ciocateu reagent method. 1ml of stem bark extract was taken and 0.5 ml of Folin-ciocalteu reagent (0.5 N) was added to it. This solution was incubated at room temperature for 15 minutes. Then 2.5 ml saturated sodium carbonate was added and further incubated in dark for 40 minutes at room temperature. Absorbance was measured at 725 nm. Gallic acid was used as standard. The total phenolic content was expressed as mg of gallic acid equivalents/g of dry weight [19, 20].

2.8 Determination of total flavonoid content
The total flavonoid content was determined by aluminium chloride method [21]. 1 ml of extract was mixed with 4 ml of water and 0.3 ml of sodium nitrite solution (5%). Then, 0.3 ml of aluminium chloride (10%) was added after 5 minutes. The solution was kept for 5 minutes and then, 2 ml of 1M NaOH was added to it. The solution was diluted to a final volume of 10 ml and absorbance was measured at 510 nm. Total falavonoid content was determined by using calibration curve of quercetin and expressed as mg of quercetin equivalents/g of dry weight.

3. Results and Discussion
3.1 Morphological studies
The bark occurred in flat pieces with thickness of 5 to 8 mm with fibrous fracture. Young fresh bark was of grey in colour while mature bark was dark greyish brown. External surface of younger bark was smooth while older bark was rough with longitudinal and transverse striations. Internal surface was smooth with light colour bearing few dark brown patches in mature bark. It had characteristic odour and non-bitter in taste. Results are shown in the Figure 1.

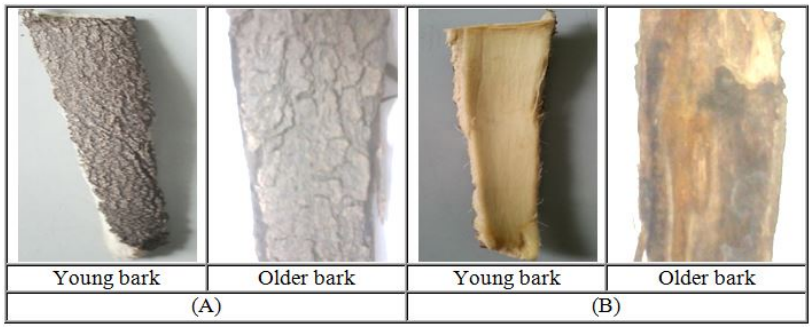

Fig 1: Stem bark of *Acacia auriculiformis* A. Cunn. ex. Benth. (A) External surface of bark (B) Internal surface of bark

3.2 Microscopic Studies

3.2.1 Transverse section of bark

The transverse section of stem bark showed periderm region consisting of cork, phellogen and phelloderm. The outermost multilayered cork cells were filled with black brown content of tannin. A broad phelloderm region was present after 3-4 layered phellogen. Phelloderm contains several layers of closely packed cells. The cortex consists of parenchyma and large number of groups of stone cells. In phloem region, sieve tubes were present with companion cells. The radially elongated medullary rays extended to phloem region were bi-seriate while in outer phloem region few rays were multiseriate. The phloem tissue was associated with phloem parenchyma and phloem fibers. Prismatic calcium oxalate crystals were also present in phloem region.

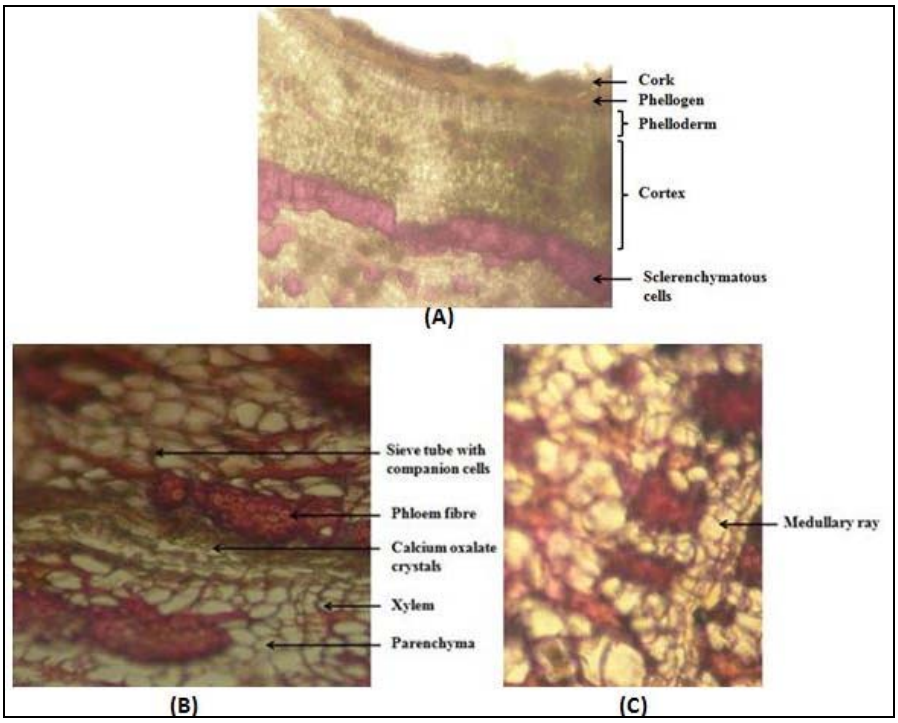

Fig 2: Transverse section of *Acacia auriculiformis* A. Cunn. Ex. Benth. Stem bark (A) Periderm and cortex region of stem bark (B) Phloem region of stem bark

3.2.2 Powder microscopy

In powder microscopy, *Acacia auriculiformis* stem bark showed the presence of parenchyma cells, sclerenchyma cells, cork cells filled with tannin, lignified fibres, medullary rays, tracheids, starch grains, prismatic calcium oxalate crystals, bordered pitted xylem vessel which are shown in figure 3.

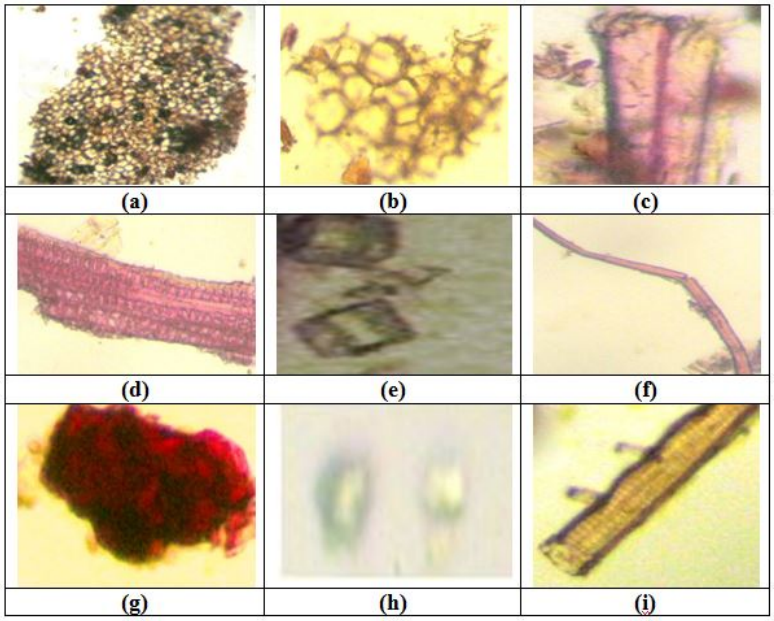

Fig 3: Powder microscopy of *Acacia auriculiformis* A. Cunn. Ex. Benth. Stem bark
(a) Cork cells filled with tannin (b) Parenchyma cells (c) Tracheids (d) Lignified fibres covered with calcium oxalate crystals (e) Prismatic calcium oxalate crystals (f) Phloem fibre (g) Sclereids (h) Starch grains (i) Xylem vessel

3.2.3 Histochemical detection

Histochemical studies showed the presence of cellulose cell wall**,** lignified cell wall, suberized cell wall, aleurone grains, starch, calcium oxalate crystals, fats and tannins. The results are shown in Table 1. The microscopic characteristics will be useful in differentiation of the plant from other related species.

Table 1: Histochemical detection of cell wall and its contents in *Acacia auriculiformis* A. Cunn. Ex. Benth. Stem bark

Test	Result
Cellulose cell wall	+
Lignified cell wall	+
Suberized cell wall	+
Aleurone grains	+
Calcium oxalate	+
Calcium carbonate	-
Fats	+
Hydroxyanthraquinones	-
Volatile oil and resins	-
Starch	+
Tannins	+

+ present; - absent

3.3 Physicochemical analysis

The results of different standardization parameters such as foreign organic matter, loss on drying, extractive values, ash values, swelling index and foaming index are given in the Table 2. These physicochemical parameters will be useful in identification of the plant stem bark even in its powdered form.

Table 2: Physicochemical characters of *Acacia auriculiformis* A. Cunn. Ex. Benth. Stem bark

S.no.	Parameter	Value
1.	Foreign organic matter	0.68 ± 0.15 %w/w
2.	Loss on drying	3.88± 0.27 % w/w
3.	Swelling index	0.00 ml/g
4.	Foaming index	Less than 100
5.	Extractive values	
	Ethyl acetate extractive value	
	Hot extraction method	8.4 ± 0.11 % w/w
	Cold maceration method	5.12 ± 0.39 % w/w
	Ethanol extractive value	
	Hot extraction method	7.2 ± 0.18 % w/w
	Cold maceration method	6.43 ± 0.08 % w/w
	Aqueous extractive values	
	Hot extraction method	6.16 ± 0.16 % w/w
	Cold maceration method	5.33 ± 0.33 % w/w
6.	Ash values	
	Total ash	11.05 ± 0.62% w/w
	Acid insoluble ash	1.33 ± 31% w/w
	Water soluble ash	2.16 ± 0.22% w/w
	Sulphated ash	9.63 ± 0.14% w/w

Values in % w/w are expressed as mean ± SEM; n=3

3.4 Elemental analysis

The elemental contents Pb, As, Zn, Cd, Cu, Hg, Fe and Mg were analysed in the powdered stem bark and were found to be in limits. The results are shown in the Table 3.

Table 3: Elemental analysis of *Acacia auriculiformis* A. Cunn. Ex. Benth. Stem bark

Metal	Concentration (ppm)
Lead	0.311
Arsenic	0.000
Copper	9.153
Cadmium	0.006
Mercury	1.515
Magnesium	8.537
Zinc	0.311
Iron	3.418

3.5 Preliminary phytochemical screening

Preliminary phytochemical analysis of *Acacia auriculiformis* A. Cunn. Ex. Benth. Stem bark showed the presence of carbohydrates, anthraquinone glycosides, saponins, phenols, tannins, flavonoids, steroids and terpenoids in different extracts. The results of preliminary phytochemical screening of stem bark extracts are given in Table 4.

Table 4: Preliminary phytochemical screening of *Acacia auriculiformis* A. Cunn. Ex. Benth. Stem bark extracts

Test	Ethyl acetate extract	Ethanol Extract	Aqueous Extract
Carbohydrates			
Molish test	-	+	+
Benedict's test	-	+	+
Fehling's test	-	+	-
Barfoed test	-	+	-
Test for pentose sugar	-	+	-
Test for hexose sugar	-	-	-
Alkaloids			
Dragondroff's test	-	-	-
Meyer's test	-	-	-
Wagner;s test	-	-	-
Hager's test	-	-	-
Anthraquinone glycosides			
Modified Borntrager's test	-	+	-
Cardiac glycosides			
Keller Killiani test	-	-	-
Legal test	-	-	-
Cynophoric glycosides			
Sodium picrate test	-	-	-
Coumarin glycosides			
Fluorescence test	-	-	-
Saponins			
Foam test	-	+	+
Flavonoids			
Vanillin HCl test	+	+	-
Ammonia test	+	+	-
Shinoda test	+	+	-
Phenols			
Ferric chloride test	-	+	+
Lead acetate test	-	+	+
Steroids and terpenoids			
Salkovaski Test	+	+	-
Libermann Burchard's test	+	+	-

+= Present, = absent

3.6 Determination of total phenolic content

The total phenolic content in stem bark extract was estimated by Folin Ciocalteu's method. Gallic acid was used as standard. The gallic acid solutions of different concentration (20-100 ppm) confirmed to Beer's Law at 725 nm with

regression co-efficient (R^2) = 0.998. The equation of standard curve is y = 0.011x + 0.085. Total phenolic content was found to be 90±0.12 mg of gallic acid equivalents/g of dry weight.

3.7 Determination of total flavonoid content

The total flavonoid content in stem bark extract was estimated by Folin Ciocalteu's method using quercetin as standard. The solutions of quercetin (20-100 ppm) confirmed to Beer's Law at 510 nm with a regression co-efficient (R^2) = 0.998. The equation of standard curve is y = 2.100x -0.196. Total flavonoid content was found to be 76±0.14 mg of quercetin equivalents/g of dry weight.

4. Conclusion

The study was performed to develop the quality control parameters of *Acacia auriculiformis* A. Cunn. ex. Benth. stem bark. The results obtained from pharmacognostical studies and preliminary phytochemical screening can be used as a diagnostic tool for the standardization of the *Acacia auriculiformis* A. Cunn. ex. Benth. stem bark to facilitate quality control and identification of the plant and to minimize the adulteration.

5. References

1. Chanda S. Importance of pharmacognostic study of medicinal plants: an overview. Journal of Pharmacognosy and Phytochemistry. 2014; 2(5):69-73.
2. Amponsah IK, Mensah AY, Otoo A, Mensah MLK, Jonathan J. Pharmacognostic standardisation of *Hilleria latifolia* (Lam.) H. Walt. (Phytolaccaceae). Asian Pacific Journal of Tropical Biomedicine. 2014; 4(12):941-946.
3. Ahmad S. Introduction of Plant Constituents and their Tests. Jamia Hamdard, Hamdard Nagar, New Delhi, 2007.
4. Kushalpa KA. Performance of *Acacia auriculiformis* in India. In: John W Turnbull editor. Advances in tropical acacia research: proceedings of an international workshop held in Bangkok, Thailand, 11-15 February, 1991. Australian centre for International agricultural Research proceedings no. 35, 189-193.
5. Singh R, Singh S, Kumar S, Arora S. Evaluation of antioxidant potential of ethyl acetate extract/fractions of *Acacia auriculiformis* A. Cunn. Food and Chemical Toxicology. 2007; 45:1216-1223.
6. Okokon JE, Jackson O, Opara KN, Emmanuel E. In vivo antimalarial activity of ethanol leaf extracts of *Acacia auriculiformis*. International Journal of Drug Development & Research. 2010; 2(3):482-487.
7. Ghosh M, Babu SP, Sukul NC, Mahato SB. Antifilarial effect of two triterpenoid saponins isolated from *Acacia auriculiformis*. Indian journal of experimental biology. 1993; 31(7):604-606.
8. Ghosh NK, Babu SP, Sukul NC. Cestocidal activity of *Acacia auriculiformis*. Journal of Helminthology. 1996; 70(2): 171-172.
9. Mandal P, Sinha Babu SP, Mandal NC. Antimicrobial activity of saponins from *Acacia auriculiformis.* Fitoterapia. 2005; 76(5):462-465.
10. Parkashi A, Ray H, Pal BC, Mahato SB. Sperm immobilizing effect of triterpene saponins from *Acacia auriculiformis*. Contraception. 1991; 43:475-483.
11. Singh S, Sharma N, Evaluation of Wound Healing Activity of *Acacia auriculiformis* A. Cunn. Stem Bark. Asian Journal of Pharmaceutical and Clinical Research, 2014; 7:204-207.
12. Singh S, Sharma N. Evaluation of *in vitro* anti arthritic activity of *Acacia auriculiformis* A. Cunn. Ex. Benth. Stem bark. World Journal of Pharmacy and Pharmaceutical Sciences. 2016; 5(2):1659-1664.
13. Kaur K, Arora S, Hawthorne ME, Kaur S, Kumar S, Mehta R. A correlative study on antimutagenic and chemopreventive activity of *Acacia auriculiformis* A. Cunn. And *Acacia. nilotica* (L) Willd ex Del. Chemical Toxicology. 2002; 25(1):39-64.
14. Arumugam S, Perumal S. Protective effect of bark and empty pod extracts from *Acacia auriculiformis* against paracetamol intoxicated liver injury and alloxan induced type II diabetes. Food and Chemical Toxicology. 2013; 56:162-170.
15. World Health Organization. Quality control methods for herbal materials. (Updated edition of quality control methods for medicinal plant materials, 1998). Malta: World Health Organization. 2011.
16. Indian Pharmacopoeia. Ghaziabad, India: The Indian Pharmacopoeia Commission, Government of India, 2007, 1.
17. AOAC. AOAC official methods of analysis. 15th ed. Association of Official Analytical Chemists. Arlington: Virginia, 1990, 84-85.
18. Hseu ZY. Evaluating heavy metal contents in nine composts using four digestion methods. Bioresource Technology. 2004; 5:53-59.
19. Kujala TS, Loponen JM, Klika KD Pihlaja K. Phenolic and betacyanins in red beetroot (*Beta vulgaris*) root: distribution and effects of cold storage on the content of total phenolics and three individual compounds. Journal of Agricultural and Food Chemistry. 2000; 48:5338-5342.
20. Siddhuraju P. Antioxidant activity of polyphenolic compounds extracted from defatted raw and dry heated Tamarindus indica seed coat. LWT - Food Science and Technology. 2007; 40:982-990.
21. Zhishen J, Mengcheng T, Jianming W. The determination of flavonoid contents on mulberry and their scavenging effects on superoxide radical. Food Chemistry. 1999; 64:555-559.